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C8-529 SPECIFICITY OF HUMAN T LYMPHOCYTES IS GENETICALLY REDIRECTED BY CHIMERIC T-BODY RECEPTORS. M. Kruger, A. Bhullar, G. Chiang, R. Goodenow, H. Gregory, P. Hamey, A. Kahrs, C. Killion, I. Krapf, C. Lundak, E. McLaughlin-Taylor, J. Reuter, E. Rodriquez, G. Sulya, J. V rnachio, and A. Williams, Gen Therapy Unit, Biotech Group, Baxter Healthcare Corporation, Santa Ana, CA 92705

A T-body is a genetically modified chimeric receptor that consists of the combining site specificity of an antibody with a defined signal transduction element. We are using T-body technology to develop a novel approach toward cancer immunotherapy. Introduction of these chimeric molecules into T cells allows the generation of T lymphocytes with antibody specificity independent of MHC restriction while maintaining T cell effector function. Chimeric genes were constructed using the antigen binding domains of monoclonal antibodies with specificity for either carcinoembryonic antigen or Her2/neu, the predominant tumor associated antigens of colon and breast cancer respectively. Single chain antibody variable regions (V_L/V_H) were linked to different signal transducing subunits (TCRβ, CD3ζ, FcRIIIγ) and cloned into retroviral vectors. We have used these vectors to introduce T-body genes into human peripheral blood T lymphocytes, tumor infiltrating lymphocytes and T cell lines as well as other cell types. Gene integration was confirmed by polymerase chain reaction (PCR) amplification and transduction efficiency was assessed by quantitative southern blot hybridization. T-body gene transcription was detected by reverse transcriptase PCR. Furthermore, these chimeric receptors were expressed on the cell surface and mediated T-cell cytokine secretion through specific recognition of antigen expressed on tumor cells. Parameters are currently being defined to optimize transduction efficiency in human peripheral blood T lymphocytes.

C8-530 ELIMINATION OF INTRAOCULAR TUMORS BY ACTIVATION OF SPECIFIC T CELLS. Bruce R. Ksander, D. Corey Gear, Eckhard R. Podack, and Peter W. Chen, Department of Ophthalmology, Harvard Medical School, Boston, MA 02114, Department of Microbiology, University of Miami Medical School, Miami FL 33136.

Activation of antigen-specific T cells requires costimulatory signals that can be provided by the interaction of B7-1 and CD28. Recent data from other laboratories indicate that tumor cells that express B7-1 directly activate CD8+ cytotoxic T cells (Tc), bypassing the requirement for CD4+ T helper cells. Our previous results indicate that immunogenic P815 tumor cells grow progressively within the immunologically privileged anterior chamber (AC) of the eye of BALB/c mice. The failure to eliminate AC tumors coincides with the failure of infiltrating pTc to differentiate further into Tc. To determine if the expression of B7-1 initiates differentiation of pTc and rejection of AC tumors, P815 tumor cells were transfected with the episomal vector pEMGNeo containing B7-1 cDNA. Either B7-1 positive, or B7 negative, P815 cells were injected into the AC and tumor growth determined quantitatively by slit lamp examination. As expected B7 negative tumor cells grew progressively. By contrast, B7-1 positive tumors were eliminated completely. Tumor rejection was T cell dependent and SCID mice failed to reject B7-1 positive tumors. Tc were recovered from the tumor-containing eye that lysed specifically P815, but not third-party EL-4 target cells. We conclude that B7-1 positive tumors initiate the differentiation of infiltrating pTc into Tc that eliminate AC tumors. These results imply that the success of immunogenic tumors within immune privileged sites may depend upon preventing APC from up-regulating B7-1 expression and inducing specific Tc. (Supported by NEI-08122).

C8-531 LIPOSOMAL TRANSFECTION OF MURINE BLADDER CANCER CELLS (MBT-2) WITH INTERLEUKIN-2 (IL-2). William A. Larchian[1,3], Warren D. W. Heston[1], William R. Fair[1], Ramila Philip[2], Mohan Philip[2] and Eli Gilboa[3], [1]Division of Urologic Surgery, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, [2]Applied Immune Science, Santa Clara, CA and [3]Department of Experimental Surgery, Duke University Medical Center, Durham, North Carolina 27710

The production of cytokine tumor vaccines has repeatedly been accomplished with transfecting agents that result in the stable and persistent expression of the cytokine. Our previous work with retroviral IL-2 transfection into the MBT-2 murine bladder cancer model, revealed stable expression of IL-2 (2 ng/1 x 10^6 cells/24 hrs) in vitro, and both protection and survival advantages in vivo. With hopes of increasing cytokine expression and eliminating the inherent concerns of using retroviral vectors, especially in the clinical setting, we pursued an alternative transfecting strategy. We studied eight different liposomes, both commercial and investigative, and determined that DMRIE/DOPE (50/50) (Vical Corporation) provided the best transfection results in our MBT-2 model. With the use of an adeno-associated viral (AAV) plasmid containing human IL-2, we generated dose (liposome/DNA 10 ul/1 ug to 80 ul/20 ug) and time (0 to 18 days) response curves. At optimal liposome/DNA ratios (40 ul/10 ug) MBT-2 cells expressed levels of IL-2 (by ELISA) in the range of 180 ng/1 x 10^6 cells/24 hrs, 3 days following transient transfection. All cells were radiated with 7000 rads 24 hours after transfection. Approximately 80% of peak levels of IL-2 expression were maintained for up to 9 days following transfection. In vivo studies analyzing this model's effectiveness are in progress. We believe that liposomal transfection in selected cell lines provides a simple, fast and highly efficient means of transient transfection for cytokine delivery.

C8-532 AAV PLASMID: LIPOSOME COMPLEXES FOR THE GENETIC MODIFICATION OF FRESH PRIMARY TUMOR CELLS: USE IN TUMOR VACCINATION FOR BREAST CANCER. Jane Lebkowski, Mohan Philip, Kim Lyerly*, Brian Clary*, Elisa Brunette, Lydia Kilinski, Deepa Murugesh, Eamonn Coveney*, Thomas B. Okarma and Ramila Philip, Applied Immune Sciences, Inc., Santa Clara, CA 95054, *Duke University, Durham NC 27710.

The use of gene modified tumor cells for the immunization of tumor bearing animals is well established. One complication to its widespread application for human therapy is the inability to efficiently establish lines from many primary tumors. To overcome this problem we have developed procedures to efficiently express transgene in primary tumor homogenates and ascites cells using adeno-associated virus (AAV) based plasmids complexed to cationic liposomes. In these procedures, the primary tumor is homogenized and single cells are collected. T cells are then depleted using an AIS CELLector CD5/8. The T depleted nonadherent fraction is collected and lipofected using cationic liposomes and plasmids containing the inverted terminal repeats of AAV for efficient gene expression. In these studies, primary lung, breast, and ovarian tumors have been transfected with plasmids containing the IL2, chloramphenicol acetyl transferase or nerve growth factor receptor genes. Tumor homogenates transfected in this manner express 500-10,000 pg IL2/10^6 cells/24 hrs after 3 days incubation. Expression of IL2 is maintained in these tumor cells for at least 7-25 days post lethal irradiation at a dose of 10,000 rads. The successful use of these procedures for vaccination protocols has now been verified using a nonimmunogenic mouse breast tumor metastasis model. These procedures will now be implemented in the gene modification of fresh breast tumors for use in immunization of patients with metastatic breast cancer.